

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT(S) : Copeland et al.

TITLE : **A MEDIUM COMPOSITION, METHOD  
AND DEVICE FOR SELECTIVELY  
ENHANCING THE ISOLATION OF  
ANAEROBIC MICROORGANISMS  
CONTAINED IN A MIXED SAMPLE WITH  
FACULTATIVE MICROORGANISMS**

APPLICATION NO. : 10/007,739

FILED : November 8, 2001

CONFIRMATION NO. : 4971

EXAMINER : Virginia Allen Portner

ART UNIT : 1645

LAST OFFICE ACTION : July 10, 2006

ATTORNEY DOCKET NO. : OXRZ 2 00025

MAIL STOP AMENDMENT  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 CFR 1.132**

Dear Madam:

The undersigned declares as follows:

1. My name is James C. Copeland, Ph.D. I am one of the inventors in the above-captioned patent application.
2. I am familiar with the subject matter in this patent application.
3. *Escherichia coli*, or *E. coli*, is known to one of ordinary skill in the art to be a facultative microbe. In other words, it can grow in an aerobic or anaerobic environment.
4. Azide is known to inhibit the growth of certain microorganisms.

5. Page 10, lines 5 and 6, of the specification, states that we found that growth of *E. coli* under anaerobic conditions is completely inhibited, or nearly so, by such poisons.

6. Page 19, line 16, to page 21, line 12, of the specification describes an experiment which shows that the growth of intact *E. coli* microbes is inhibited by azide. As described there, sodium azide in the amount of 0.1 mg/ml and sterile membrane fragments obtained from *E. coli* were added into tubes. The tubes were then inoculated with various anaerobes and facultative microbes, including *E. coli*. The results of the experiment showed that the anaerobes grew, as indicated by turbidity. However, *E. coli* did not grow, as indicated by the lack of turbidity.

7. This experiment showed that the growth of intact *E. coli* microbes is inhibited by azide.

8. This experiment also showed that the sterile membrane fragments obtained from *E. coli* were not inhibited by azide.

9. Because intact *E. coli* microbes are inhibited by the respiratory inhibitor azide, it would be expected by one of ordinary skill in the art that respiratory enzymes on sterile membrane fragments obtained from *E. coli* would also be inhibited by azide.

10. Hence, it was highly unexpected that the respiratory enzymes on the sterile membrane fragments obtained from *E. coli* continued to function in the presence of azide, as exhibited by the growth of anaerobes.

11. Page 10, lines 8 to 11, also state that the inventors unexpectedly found that the respiratory enzymes bound to the membrane of *E. coli* were not inhibited by poisons or inhibitors of the respiratory electron transport system.

12. Page 21, line 14, to page 22, line 21, of the specification describes an experiment to determine the effect of the concentration of azide on the function of sterile membrane fragments obtained from *E. coli*. Three azide concentrations were tested, 0.01 mg/ml, 0.1 mg/ml, and 1.0 mg/ml. The activity of the sterile membrane fragments was unchanged in all three concentrations over 90 minutes.

13. I am familiar with the subject matter in Merad. In particular, I am aware that Merad used 0.1% azide to inhibit facultative microbes.

14. I am familiar with the subject matter in U.S. Patent No. 4,476,224 to Adler. In particular, I am aware that Adler used membrane fragments derived from *E. coli* to create an anaerobic environment.


15. I am familiar with the subject matter in U.S. Patent No. 5,830,746 to Copeland, one of my earlier patents. In particular, I am aware that this patent discloses the use of membrane fragments derived from *E. coli* to create an anaerobic environment.

16. I am familiar with the subject matter in U.S. Patent No. 5,405,773 to Fung. In particular, I am aware that Fung used membrane fragments derived from *E. coli* to create an anaerobic environment.

17. I believe that because one of ordinary skill in the art would expect azide to inhibit the growth of *E. coli*, one of ordinary skill in the art would not be motivated to combine Merad with Adler, Copeland, or Fung and use azide in conjunction with membrane fragments derived from *E. coli*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

By   
Printed Name: James C. Copeland, Ph.D.  
Date: 02/06/07